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STIMULATION OF ENDOPARASITOID EGG DEVELOPMENT BY A FAT BODY CELL LINE: ACTIVITY AND CHARACTERIZATION OF FACTORS THAT INDUCE GERM BAND FORMATION AND HATCHING

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SUMMARY

The capacity of a fat body cell line to induce embryogenesis in an endoparasitoid, *Microplitis croceipes* was compared with host fat body. Pregerm eggs were dissected from the host, *Helicoverpa zea*, and incubated in IPL-52B medium preconditioned for 24 hrs with the cell line IPLB-LdFB derived from the gypsy moth, *Lymantria dispar*, whole fat body tissue from the host *H. zea* or a defined lipid concentrate. The response of the eggs to the conditioned media was dose dependent. The cell line and host fat body tissue induced both germ band formation and hatching, while lipid concentrate only promoted developed to the germ band stage. The active material(s) that induced germ band development was dialyzable (<10 K), whereas the components(s) that stimulated hatching was nondialyzable (>10 K). The active materials in both media were resistant to trypsin and heat.

INTRODUCTION

The endoparasitoid, *Microplitis croceipes* (Cresson) (Hymenoptera:Braconidae), is an important parasite of the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.) in the southern region of the United States (8,12). Because of the parasitoid's potential use as a biological control agent for *Heliothis spp.* in pest management programs, considerable attention has been directed toward development of an artificial culture rearing medium to replace the host and hopefully to reduce costs for mass rearing of the parasitoid (6).

Greany (6) demonstrated that egg development *in vitro* could be stimulated by exposure to medium preconditioned by fat body dissected from *H. zea* larvae. Subsequently we showed that a fat body cell line could be used to precondition medium that promoted embryogenesis of the parasitoid (2). Although growth factors that induce embryonic development have not yet been isolated from insect tissues (3), we suspect that there are factors that induce embryonic development similar to those reported in vertebrate systems. Vertebrate growth factors that induce differentiation of tissues in the vertebrate embryos include vimentin which induces cleavage in the fertilized egg in the African clawed frog, *Xenopus laevis*, and activin (transforming-growth factor-B), which induces undifferentiated cells in the blastula to become ectoderm, mesoderm, or endoderm, depending on the concentration of the factor (1,11). Finally, retinoic acid which binds to a putative zinc finger receptor polypeptide, was recently proposed as a fundamental morphogen in the developing vertebrate embryo (7,13).

In our laboratory, we have sought to determine the activity and nature of the embryogenesis-promoters that are released by fat body cells. The experiments described here provide a detailed analysis of the response of the parasitoid egg to fat body cell line-conditioned medium. Also we report some of our early efforts to characterize the nature of the factor(s).

MATERIALS AND METHODS

Host and parasite colony maintenance. The host species, *H. zea*, was mass-reared in our laboratory according to previously described procedures (9). Rearing procedures for *M. croceipes* were as previously reported (4).

Basal medium preparation. Goodwin's IPL-52B medium, a chemically defined basal culture medium (5), was prepared as described earlier (3) and was adjusted to ca. 340 mOsm at pH 6.4 and supplemented with 2 mg glutamine per ml. Abbreviations of the medium preparations are the following: fat body cell line conditioned medium (FBCELLCM), and fat body tissue conditioned medium (FBTCM).

Cell culture and preconditioned media. The cell line developed from gypsy moth fat body, IPLB-LdFB, was maintained as described by Lynn et al. (10). Medium was conditioned with the cell line as described earlier (2). Briefly, cell counts were made with a hemocytometer, and the cells were then allowed to settle and the media removed. Cells were resuspended at a concentration that equaled 1 X 10^4 cells/0.1 ml to condition the medium. Dilutions of this original cell count were made to obtain a dose response curve.

Fat body preconditioned media. Twenty mg (wet wt.) of fat body was used to condition 100 μl of medium at 26°C for 20 hrs. In one experiment, medium was diluted with unconditioned medium to obtain dose response data. We compared the developmental response obtained with medium that was pre-conditioned with the fat body cell line versus host larval fat body. To do this we determined the percentage of fat body and blood volumes at various larval weights (450-700 mg/larva) to compare the bioassay response with larval equivalencies of fat body. Fat body was dissected from fifth instar larvae as described earlier (2) and weighed to determine the percentage of fat body per larva. The blood volume was estimated by injecting 30 fifth instar larvae with 1 μl Ringer's solution containing [H^3]-inulin (1.33 X 10^5 dpm/μl; New England Nuclear, 175.5 mCi/g) and counting 10 μl samples to determine the dilution effect after 5 and 18 hrs.

Defined lipid concentrate. A chemically defined lipid concentrate from GIBCO was incubated in IPL-52B medium at concentrations of 0.1, 0.5, 1.0, 5.0 and 10% for 24 hrs before bioassay.

Collection of eggs and bioassay. Details of the egg collection procedure and bioassay were as previously described (4). Briefly, parasite eggs were dissected from third instar host larvae that were exposed to 3-5 day old female wasps for a 2 hour period, the eggs thus being less than 2 hours old. The eggs were rinsed 5 X in IPL-52B, then 5 eggs were transferred to 100 μl of filter sterilized test medium. Each treatment of 5 eggs per 100 μl of test medium was replicated 3-9 X for a total of 15-45 eggs. Each bioassay included a control containing unconditioned medium. In addition, fat body conditioned medium at 20 mg/ml was also run as a check to insure that the eggs were capable of responding in the bioassay. Development was observed and recorded daily for 7 days at 26.6°C. Germ band formation was selected as a marker for early egg development. Data are presented as the percentage of eggs that attained germ band stage and that hatched into first instar larvae.

Dialysis. Cell line and fat body preconditioned media were dialyzed in a six-cell equilibrium chamber (MRA, Boston, MA). Conditioned medium was placed in one chamber and separated by a 12 K cut-off membrane from an adjoining chamber containing unconditioned medium. The unit was placed on a shaker for 4 hours at 23.5°C. In addition, conditioned medium was also dialyzed using a pressure flow cell (Omegacell, 10 K membrane, Filtron Technology, Clinton, MA). Two ml of conditioned medium was concentrated to 0.2 ml and then brought to the original volume with medium. The dialysate and the resultant elutant (<1.8 ml) were bioassayed.

Heat treatments. Two-hundred μl of each of fat body and cell line conditioned medium were held at 60°C for 5 and 30 min and at 100°C for 5 min, and then bioassayed.

Protease sensitivity. Five-hundred µl of conditioned medium was incubated with 50 µl of a 2.5% filtered sterilized solution of trypsin (Sigma, St. Louis, MO) for 30 min at 37°C. After incubation, 50 µl of a 2% solution of trypsin inhibitor (Sigma, St. Louis, MO) was added to the medium. Controls consisted of unconditioned medium; conditioned medium; conditioned medium + trypsin; conditioned medium + inhibitor. At the end of the incubation, trypsin activity was measured in each of the treatments by adding a 1 mM alpha-p-Tosyl-L-arginine methyl ester (TAME, Sigma, St. Louis, MO) to medium diluted with unconditioned medium (up to 1:10) and measuring the increase in absorbency at 247 nm with time.

RESULTS AND DISCUSSION

We obtained dose response data for both cell line and fat body conditioned media. Figures 1A and 1B show the percentage of pregerm eggs that attained germ band stage and that hatched after incubation in dilutions of FBTCM and FBCELLCM. The highest concentration of fat body used to precondition the medium was 20 mg or 0.2 larval equivalents/0.1 ml (Fig. 1A).

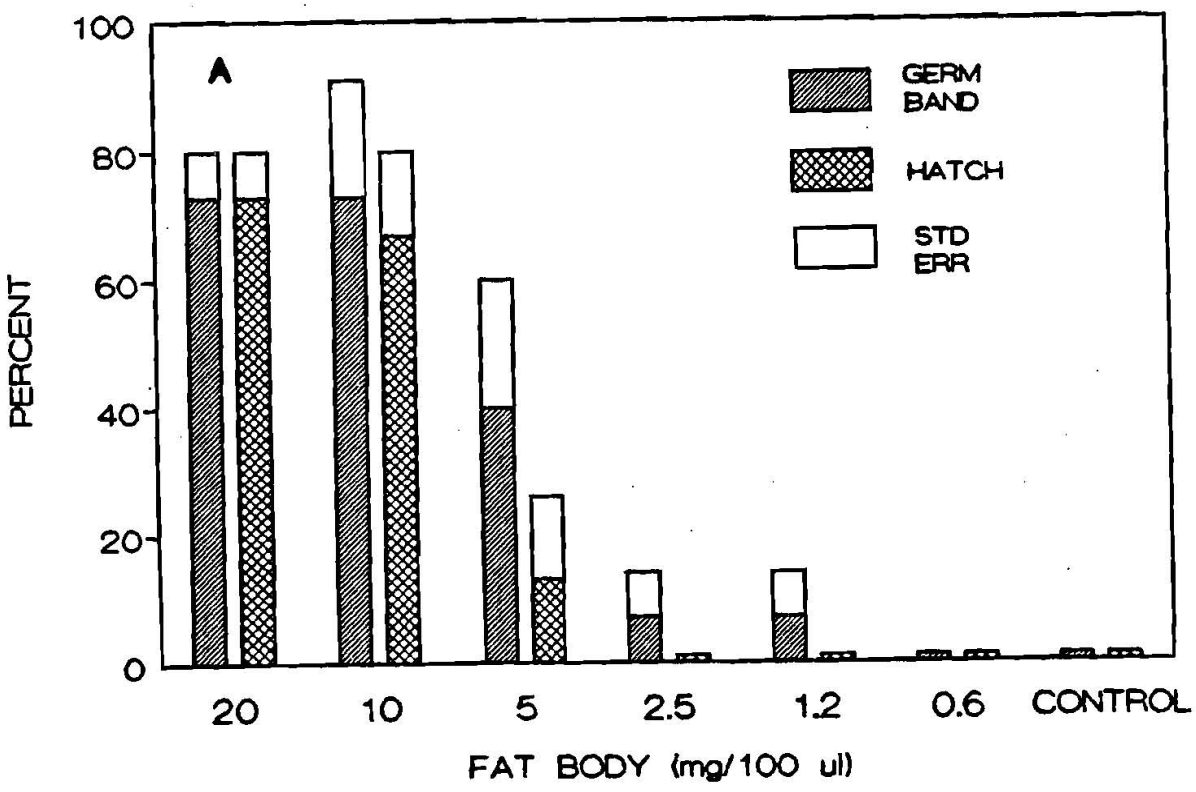

Fig. 1A. Percentage of pre-germ band eggs attaining germ band stage and hatching in IPL-52B medium pre-conditioned for 24 hrs with fat body from host larvae.

Approximately 75% of the eggs treated with 10 mg of fat body reached the germ band stage. By comparison, the cell line was nearly as effective as the fat body tissue in stimulating the eggs to develop to germ band stage in conditioned medium, with a maximum of about 60% of the eggs reaching the germ band stage (Fig. 1B).

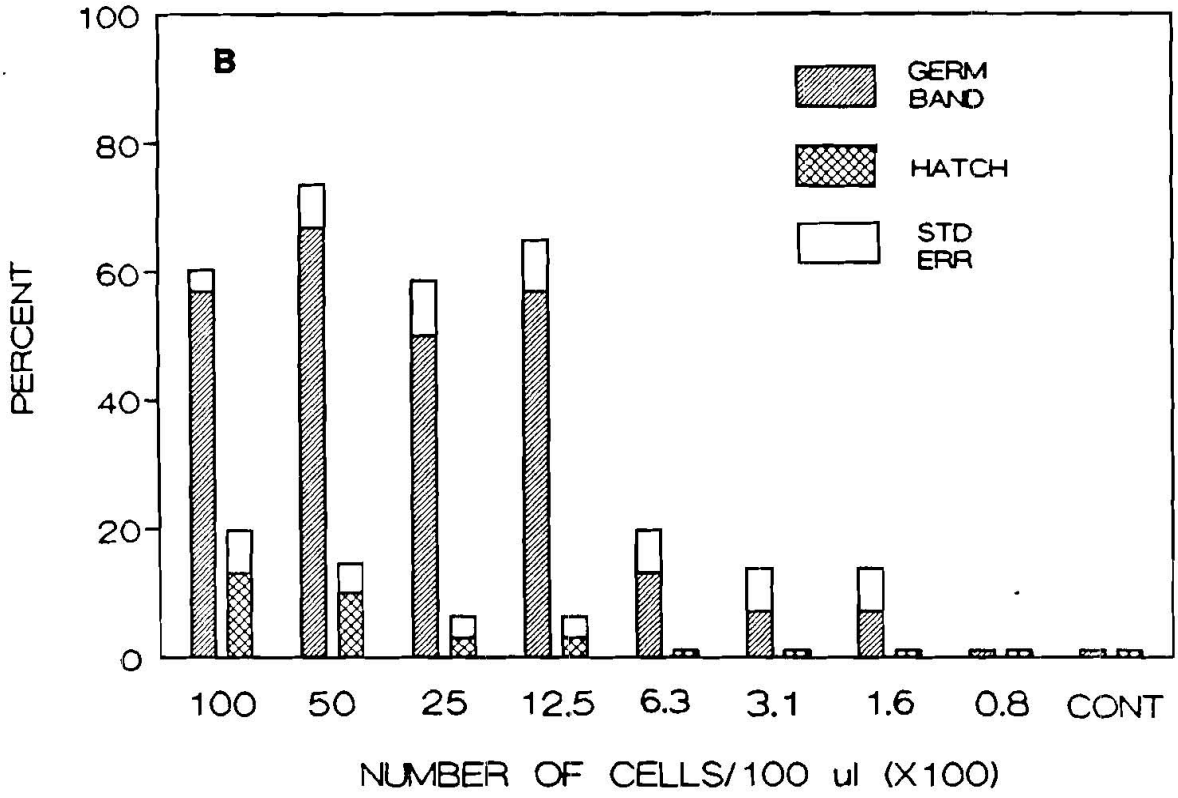

Fig. 1B. Percentage of pre-germ band eggs attaining germ band stage and hatching in IPL-52B medium pre-conditioned for 24 hrs with fat body from host larvae.

However, medium conditioned with LdFB cells induced hatching in about 15% of treated eggs compared with about 65% for eggs in medium conditioned with host fat body (Figs. 1A and 1B). This difference might result from the use of a cell line derived from a non-host species with regard to the parasitoid. Most importantly, these experiments demonstrated that the LdFB cell line is unique in stimulating hatching as well as germ band formation, while earlier studies (2) indicated that a cell line derived from *Trichoplusia ni* imaginal discs (IAL-TND1) and a medium (Excell 400) primarily composed of lipids and low protein content (<1 mg) stimulated germ band formation only. Subsequent tests with a defined lipid concentrate (GIBCO) revealed that these lipids stimulated germ band formation but did not promote hatching (Fig. 2). These results suggest that the nature of germ band stimulating material released into the medium by both the fat body tissue and cell lines may be lipoidal in nature.

We attempted to characterize the nature of the factors by several procedures, including dialysis, heat treatment and exposure to protease. The effects of various treatments on the germ band and hatch promoting activity of the FBTCM and FBCELLCM are shown in Table 1. Equilibrium dialysis indicated that the material in the FBTCM that stimulated germ band formation consisted of a dialyzable molecule that could pass through the 12 K membrane and therefore was less than 12 KDa in mass. Similarly, although the overall activity was lower, the material in the FBCELLCM was also found in the lower molecular mass compartment. This indicated that the cell line released a component in the <12 KDa fraction that stimulated germ band formation. In contrast, the >12 K fraction stimulated more eggs to hatch than the <12 K fraction, with both the FBTCM and FBCELLCM. These results indicate that a molecule with a molecular mass greater than 12 KDa was responsible for stimulating egg hatch. The equilibrium dialysis studies were confirmed with dialysis using a pressure flow cell, although the embryogenesis-promoting activity was lower.

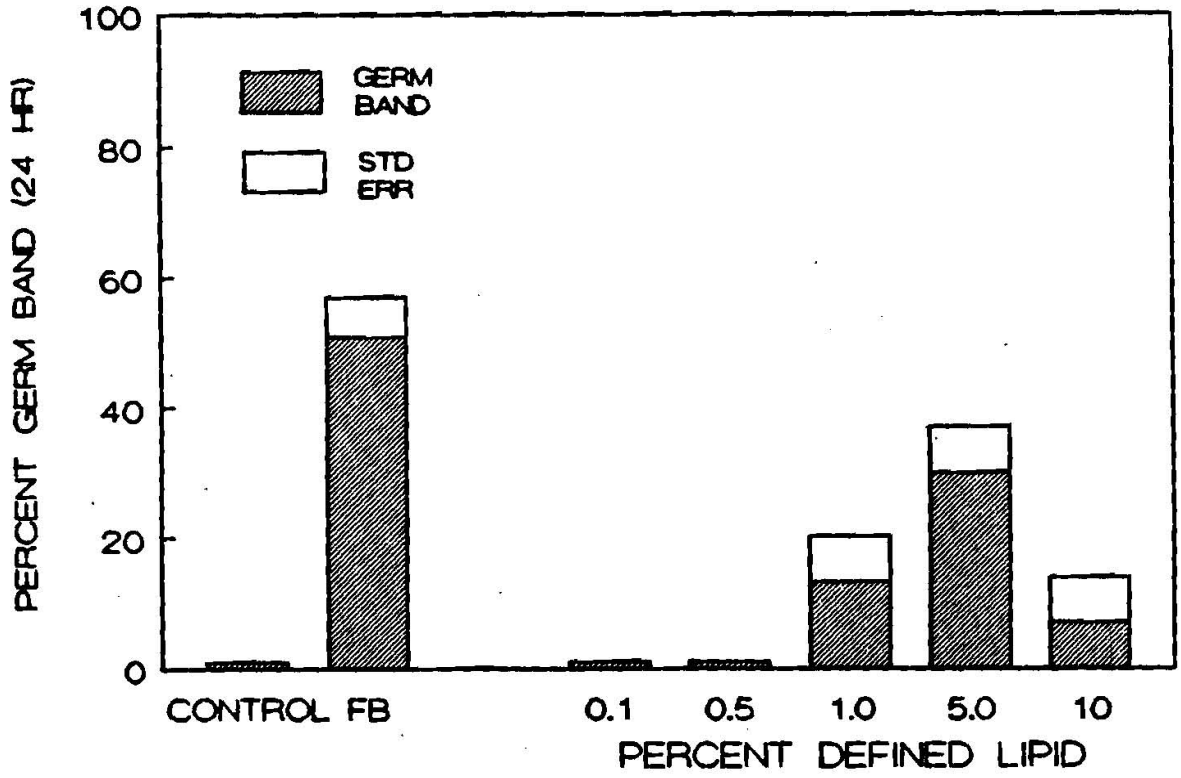

Fig. 2. Percentage of pre-germ band eggs attaining germ band stage and hatching in IPL-52B containing a chemically defined lipid concentrate.

Heating both the FBTCM and FBCELLCM for 5 min at 100°C revealed that both the germ band and the hatch factor were not heat labile because no loss of activity was observed. In addition, treatment of the media with trypsin indicated resistance to the enzyme. These results considered together suggest that the active materials are not proteineaceous.

TABLE 1
EFFECTS OF VARIOUS TREATMENTS OF FBTCM AND FBCELLCM ON PARASITOID EGG DEVELOPMENT

Treatment[a]	FBTCM		FBCELLCM	
	% germband	% hatch	% germband	% hatch
Control (medium)	0	0	0	0
Preconditioned medium	55.3±8	17.3±6	35.8±7	11.8±5
Dialysis:				
Equilibrium				
<12K	40±8	5±5	13±10	0
>12K	30±6	25±10	20±13	13±3
<12K + >12K	25±9	20±8	13±6	3±3
Pressure flow cell				
<10K	23±6	3±3	23±6	3±3
>10K	28±6	9±4	28±6	9±4
Heat				
100°C, 5 min	73±7	13±7	17±8	13±7
Trypsin				
Inhibitor	73±7	47±24	40±23	7±7
Inhibitor + Trypsin	73±18	47±18	27±7	20±20

[a]Data represent mean ± standard error.

In summary, our results show that there are at least two different substances produced by the fat body cell line and host fat body tissue that are required for egg development, a germ band stimulating factor and a factor that promotes hatching. There is a dialyzable (<10 K) factor that induces germ band development and a nondialyzable factor (>10 K) that induces hatching. The active material is resistant to trypsin and is heat resistant. Finally, tests with a defined lipid concentrate containing fatty acids (from GIBCO) induced germ band formation but not hatching. Whether or not the germ band-promoting material released by the cell line is lipoidal in nature remains to be determined.

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